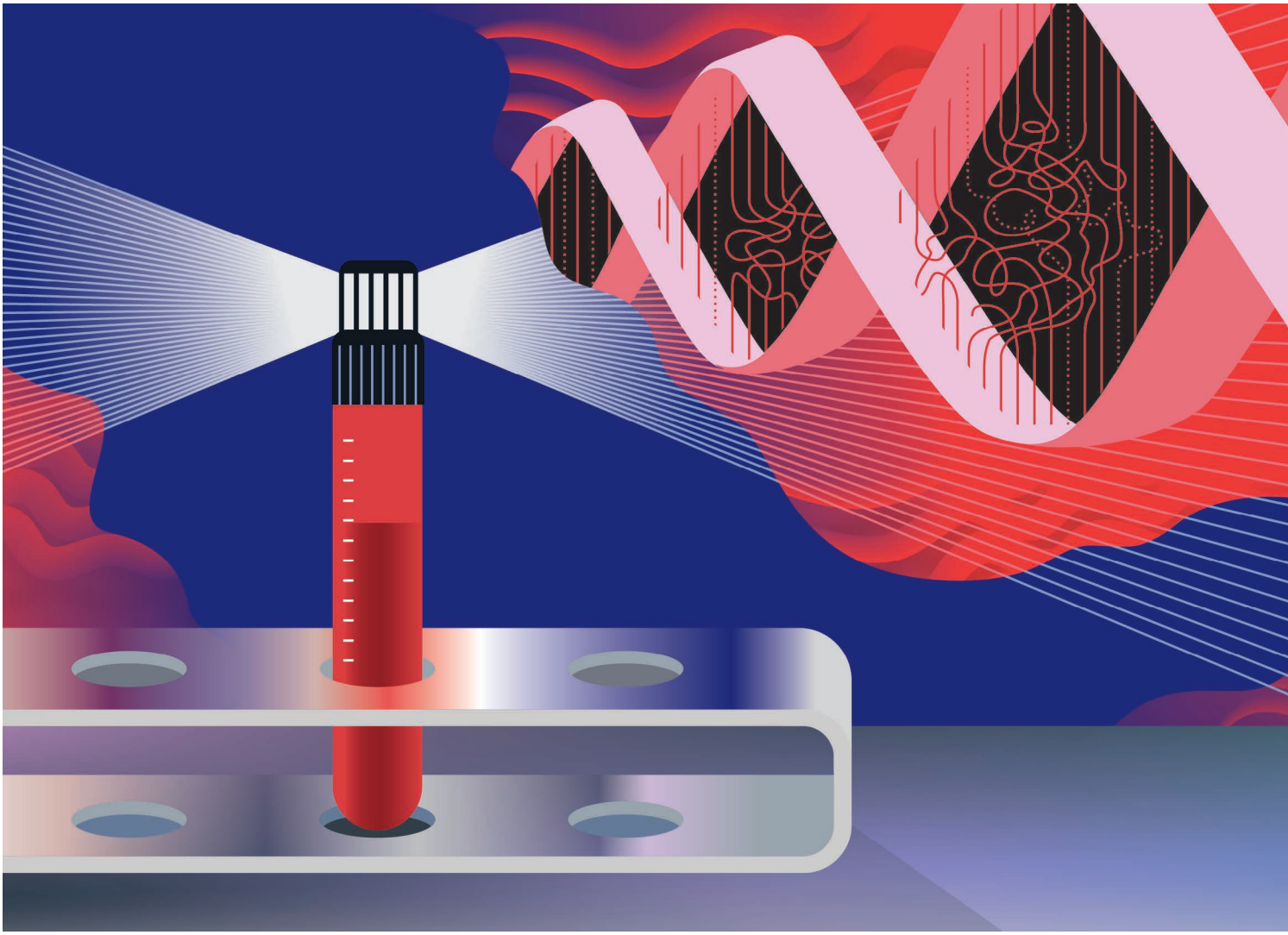


ILLUSTRATION BY ANA KOVA

DIAGNOSTICS

Know thy enemy

Are liquid biopsies, based on tumour DNA circulating in the blood, poised to improve lymphoma therapy?

BY LIAM DREW

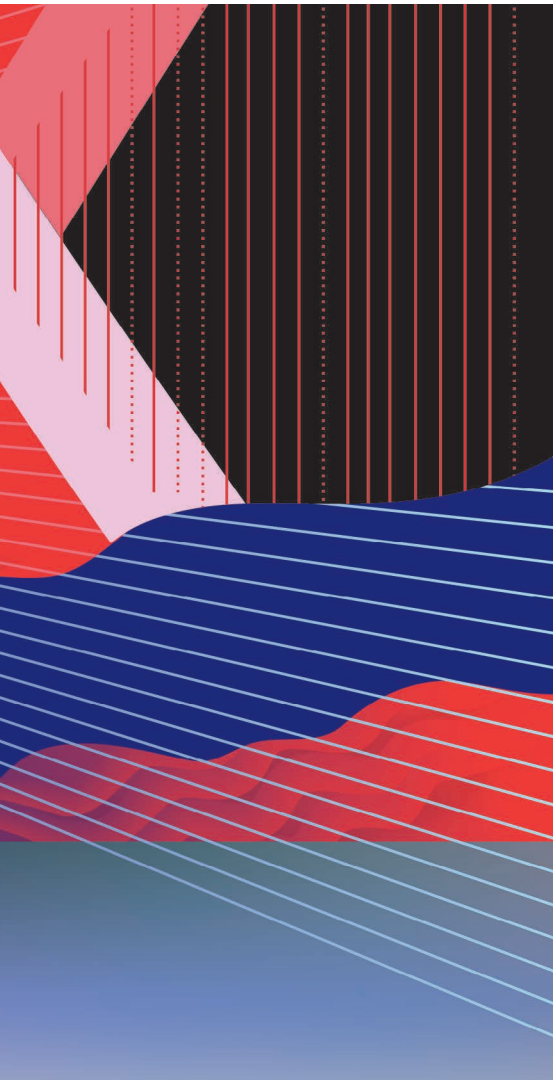
"You get a lot of information, almost immediately after you start treatment," says oncologist Mark Roschewski, talking about a blood test that can diagnose lymphomas, monitor their response to treatment and detect whether these cancers recur. "We've looked after a week and two weeks," he says, "and can predict clinical outcomes that happen five years down the road."

The test, which Roschewski is helping to develop at the US National Cancer Institute in Bethesda, Maryland, is based on the discovery that lymphomas leak significant amounts of fragmented genomic DNA into a patient's bloodstream. Work over the past few years has shown that the abundance of circulating tumour DNA (ctDNA) is proportional to tumour size, and that the DNA can be sequenced to observe the mutations underlying the cancer. Advancements in DNA detection and analysis technologies now mean that 'liquid biopsies' using a patient's blood could become widespread.

Advocates of liquid biopsies argue that they can provide crucial insights into how a lymphoma is behaving. Such information could improve clinical outcomes by enhancing decisions made regarding a patient's clinical care. Today, that care is typically guided by the results of a needle biopsy of the solid tumour at diagnosis, then through and after treatment by positron emission tomography (PET) or computed tomography (CT) scans. Roschewski and other scientists say that liquid biopsies are not only safer and more convenient than these other ways of tracking lymphomas, they are also more informative. For example, studies have indicated that measuring the levels of ctDNA can more accurately reflect the growth or shrinkage of the cancer than currently used scans[1].

But the data backing up the ability of liquid biopsies to deliver useful insights are from retrospective analyses, not current clinical usage. Studies so far have analysed blood samples that were periodically taken from patients as they completed routine treatment. Then, later on, researchers examined the samples to determine how well the ctDNA data correlated with information from biopsies and scans, as well as with various clinical endpoints, such as remission, relapse and death. So when Roschewski says he can predict what will happen to a patient in five years, he is referring to historical cases. To move liquid biopsies into standard oncology practice, it must be shown that they can change patient management — and improve outcomes.

"You can learn what happens at the end very early. And do that with very high precision:



take the blood, profile the mutations and get a number that accurately measures disease burden," says Ash Alizadeh, an oncologist at Stanford University in California who has worked on lymphoma genetics for more than 20 years. "What we don't know is if learning that information early — or learning bad news early — can be immediately actionable."

The potential of liquid biopsies is enticing, but there is no guarantee of success. "I'm very, very excited by the results," Alizadeh says. "But clinical medicine is a tough business in terms of improving upon the historical standard."

THE CHALLENGE

Diffuse large B-cell lymphoma (DLBCL) is one of the most common forms of lymphoma — and the form in which liquid biopsies have been explored most thoroughly. This variant of the disease affects around 7 people per 100,000 each year in the United Kingdom and United States, and, as is true with most lymphomas, people with DLBCL typically present with swollen lymph nodes and non-specific symptoms, such as fatigue or night sweats. Normally, only after doctors have ruled out infections and other more common complaints do they even request a biopsy, which is the current gold standard for diagnosis.

Although DLBCL is a haematological cancer, the cancerous B cells do not typically circulate in the blood. Instead they remain — at least initially — in the lymphatic system, where tumours can become large. "I see patients in my clinic who have two litres of tumour in their abdomens," says Alizadeh. "They are very highly proliferative tumours — and can be a pretty rapidly fatal disease if left untreated."

Once diagnosed, most patients undergo six cycles of combination immunochemotherapy known as R-CHOP. This is a cocktail of five drugs: four chemotherapy agents (collectively known as CHOP) and an antibody, rituximab, that binds to a unique B-cell surface marker to trigger cell death. CHOP alone has been used since the early 1990s to successfully treat about 55% of patients. The addition of rituximab in the early 2000s increased remission rates to higher than 74%.

For many patients, R-CHOP is a cure, permanently removing the cancer. But for others either it fails to lead to remission or initial remission is followed by relapse. For people in either of those situations, the odds of survival dip considerably. Second-line treatment is a more aggressive salvage chemotherapy, which requires removing stem cells from the patient's bone marrow and then returning them after chemotherapy so that they can form new blood cells. However, only 1 in 4 patients show a response to this treatment. In May, the US Food and Drug Administration approved the use of chimeric antigen receptor T-cell therapy (CAR-T therapy) as a third-line treatment for DLBCL (see page S42). This procedure entails removing a sample of a patient's T cells and genetically engineering them to express a receptor for a B-cell antigen. Then the T cells are injected back into the patient to attack the tumour.

At present, if a DLBCL is diagnosed at an advanced stage, the survival rate at five years after diagnosis is 50%. And for people with earlier-stage tumours, the five-year survival rate is 65–70%.

"You can learn what happens at the end very early. And do that with very high precision."

There are a number of ways in which liquid biopsies might improve survival. They could detect the disease earlier, provide insights into the tumour's genetics and indicate relapse or responses to therapy.

Each potential application, however, "places different demands on the technology", says Anna Schuh, director of the Molecular Diagnostics Centre at the University of Oxford, UK. But, she stresses, "the question really, with respect to people who are treating lymphoma, is how liquid biopsies are going to change your clinical management."

No one fully understands the processes that lead to high amounts of ctDNA in the blood. It seems to result from the increased rate of cell death that accompanies the rapid cellular proliferation inside tumours — the dying cells release fragments of genomic DNA that enter the bloodstream. These fragments are distinguishable from other circulating DNA by genetic features that are unique to cancer cells, and they are digested by enzymes in the blood within hours. Consequently, ctDNA gives almost real-time information about a tumour.

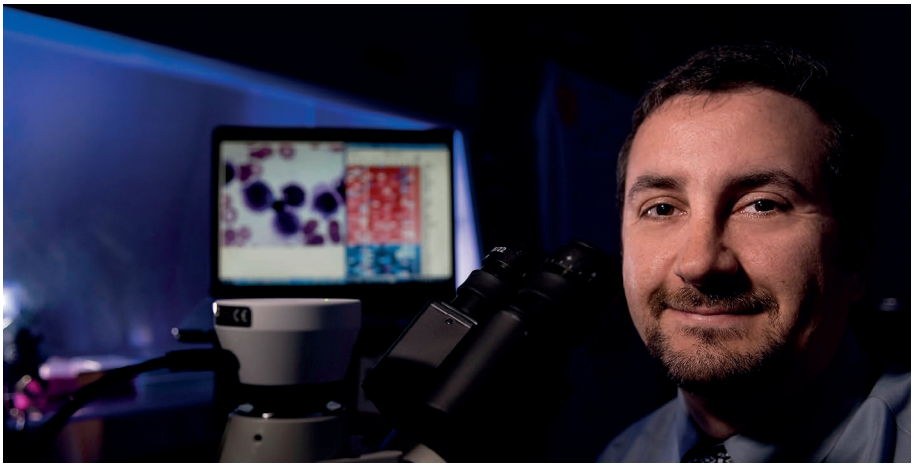
A UNIQUE SIGNATURE

A form of ctDNA analysis that is specific to lymphomas involves tracking the level of just one telltale gene — but the gene in question varies from patient to patient. After infection by bacteria or viruses, the body retains a small number of the lymphocytes (white blood cells) that responded to the infectious agent in case it recurs. These cells express a unique immunoglobulin receptor on their surface that is specific to that agent. Therefore, when a B or T cell turns malignant, all of its cancerous progeny express the same unique immunoglobulin receptor. This receptor has nothing to do with the lymphocytes becoming cancerous but it can serve as a tumour's identification tag. The gene's sequence is determined from the standard biopsy of the tumour at diagnosis and then used to spot fragments of ctDNA in the blood.

Roschewski — supported by the biotechnology company Adaptive Biotechnologies in Seattle, Washington — has used this approach to study the relationship between circulating immunoglobulin DNA and treatment outcomes in people with DLBCL. In a 2015 paper looking at 126 people[1], he and his colleagues showed that in blood samples taken after two treatment cycles, the levels of ctDNA partially indicated which patients' lymphomas had progressed further. More impressively, when monitoring people in remission, those who had detectable levels of ctDNA in their blood were more than 200 times more likely to relapse.

Alizadeh has also published on this technique[2]. He notes that in both his and Roschewski's studies, a disease signal could be detected in the blood of only a subset of patients at the time that their cancer was diagnosed. Additionally, the immunoglobulin signal became undetectable in most patients after just one cycle of therapy, even in people whose disease ultimately relapsed.

Alizadeh has therefore focused on developing a second approach, which has also been adopted by Davide Rossi, a haematologist at the Institute of Oncology Research in Bellinzona, Switzerland. This technique is called cancer personalized profiling by deep sequencing (CAPP-Seq). It uses DNA probes that are designed to bind to fragments of circulating DNA if those fragments contain any of the multiple mutations that have been catalogued as contributing to DLBCL. Rossi has had success using probes that survey a panel of 59 genes, but now he uses 133. Alizadeh's group currently examines about 300 genes to



STEVE FISCH/STANFORD SCHOOL OF MEDICINE

Ash Alizadeh at Stanford University in California is developing a system to monitor tumour DNA in blood.

maximize the number of mutations tracked.

Studies published in the past two years from both groups[3,4] have found that the mutations detected in ctDNA mostly match the mutations found by genotyping tissue taken directly from the tumour by a needle biopsy. These results not only give confidence that the technique reliably reports on the biology of the tumour, but also show other potential advantages over current techniques. For example, by looking at a series of blood samples taken regularly as patients moved through treatment, researchers saw that new mutations emerged in certain people who did not achieve remission — including mutations that might have accounted for resistance to R-CHOP. Such real-time genetic information about how a tumour evolves could be harnessed to select targeted therapies for patients with specific mutations, and to change those treatments or add other ones if further mutations emerge.

However, although therapies targeted at specific mutations occurring in subtypes of DLBCL are being actively pursued, none has yet reached the clinic. Therefore, using the genetic information in ctDNA to select a mutation-specific agent will only be possible if and when such treatment options become available. And although liquid biopsies might aid the selection of patients for future trials of such therapies, Schuh warns that "it then becomes a question of whether you're testing the liquid biopsy or the drug".

Instead, the current consensus is that ctDNA works well for monitoring disease and that, consequently, its most likely route to the clinic will be in identifying which patients are benefitting during R-CHOP treatment and which are most likely to have their disease recur. Overall levels of ctDNA, as assessed using CAPP-seq, correlate well with disease stage and tumour volume. More strikingly, when looking at blood samples taken after the first and second rounds of R-CHOP, patients who went into complete remission showed early, drastic decreases in ctDNA levels[5]. None of the patients who failed to show a molecular response during therapy went into complete remission. "If you still detect mutations at the end of therapy in the blood," says Rossi, "it is 100% sure that the patient is going to relapse."

Tracking ctDNA thus seems to offer a highly sensitive means of monitoring tumour size, spread and regrowth. Currently, such monitoring is typically done using PET or CT scans. However, when used during therapy, such scans are prone to giving false-positive readouts due to inflammation associated with chemotherapy[6]. And they cannot be used too frequently because of the cumulative radiation exposure.

"If you still detect mutations at the end of therapy in the blood, it is 100% sure that the patient is going to relapse."

Studies of blood samples taken between remission and relapse have found that detectable ctDNA returns months, and sometimes years, before clinically observable relapse occurs — a phenomenon Roschewski calls molecular relapse, which he contends should be responded to as quickly as possible. The resistance he has encountered to this proposal, he says, is based on concerns about needless treatment of false-positive results if not all ctDNA is predictive of tumour regrowth. And research across various cancers suggests that earlier treatment of relapse is not necessarily more successful. These question marks over the accuracy and usefulness of early detection by liquid biopsies are likely to be resolved only by a clinical trial.

Such a trial might take a large number of people with DLBCL and randomize them to receive treatment on molecular relapse or on clinical relapse. Roschewski says he would be uncomfortable knowing that someone had detectable levels of ctDNA but wasn't being given treatment, although ultimately this seems comparable to a patient receiving a placebo control when a promising medicine is tested.

As for ctDNA guiding early treatment choices, Alizadeh concedes that the trials will be complex. Still, he says, the richness of information that liquid biopsies offers cannot be ignored. He suggests that when the ctDNA indicates a patient is not responding well to R-CHOP — and therefore will not achieve remission — clinicians might wish to switch sooner to salvage chemotherapy or CAR-T therapy. And conversely, he says that if ctDNA is absent after one or two R-CHOP cycles, the clinician might wish to spare the patient the full six cycles and thus avoid the associated toxicities.

CANCER VERSUS ONCOLOGIST

On the central question of how liquid biopsies might be used in the clinic, Alizadeh thinks that rather than replacing any current source of diagnostic or prognostic information, they should be integrated with all possible resources. The key issue for Alizadeh is that an oncologist must be constantly asking, 'is what I'm currently doing maximizing the chances of my patient being cured?' If the answer is 'no, the data are showing my patient isn't responding to what I'm doing', then the oncologist should change to a procedure that might increase survival odds.

Alizadeh and David Kurtz, an oncologist in his group, are leading the development of an automated system that will combine constantly updated clinical information, imaging results and ctDNA to estimate the most likely outcome of a treatment strategy. Alizadeh and Kurtz liken the idea to a sports statistician monitoring a football game as it plays out, adjusting — in real time — the likelihood that either side will win. From an initial prediction based on the teams' respective qualities, the odds are updated throughout the game as the score changes, as players get injured and as tactics shift.

In such an analogy, cancer is on one side of the playing field, the oncologist on the other, each making moves that might end or save the patient's life. Alizadeh's system could tell an oncologist when their odds of winning are slipping, such as if a scan is troubling or ctDNA levels are going up. It could then indicate which change of tactics — such as a replacement therapy — might improve the odds.

To an extent, this happens already: oncologists always observe their patients and respond to what they see. But Alizadeh thinks that the degree of prognostic insight provided by ctDNA — seeing much earlier, for instance, whether R-CHOP will work, and detecting relapse earlier — will help tip the balance further in favour of the oncologists and their patients. If so, liquid biopsies might be likened to a decisive glimpse of the opponent's playbook. Future trials will determine whether doctors, in response, can make the moves that will enable them to win. ■

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